

Steroidal Sapogenins. XXXV. Gentrogenin (Botogenin) and Correllogenin, New Sapogenins from *Dioscorea spiculiflora*^{2,3,4}

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Two new ketonic sapogenins, gentrogenin (botogenin) and correllogenin, have been isolated from the tubers of *Dioscorea spiculiflora*. Gentrogenin was converted to diosgenin and hecogenin; and correllogenin, to yamogenin and sisalagenin. Accordingly, gentrogenin must be 20 α , 22 α , 25 β -, and correllogenin 20 α , 22 α , 25 β -spirost-5-en-3 β -ol-12-one.

Side chain degradation of gentrogenin gave 5,16-pregnadien-3 β -ol-12,20-dione which was converted to 5-pregnen-3 β -ol-12,20-dione, allopregnan-3 β -ol-12,20-dione, and allopregnan-3,12,20-trione. The properties of gentrogenin, correllogenin and the various pregnene and allopregnane derivatives, differed markedly from values previously presented by Marker for botogenin, neobotogenin, and various side chain degradation product.

Some years ago Marker and Lopez reported the isolation of a new sapogenin from *Dioscorea mexicana* which they called botogenin.⁵ It was characterized as 12 keto-diosgenin by conversion to diosgenin and hecogenin. Since such a sapogenin would have been a desirable cortisone precursor, we were alert for it during the screening of a large number of *Dioscorea* species,^{6a,b,c} but with negative results. Recently we isolated two isomeric 12 ketonic

sapogenins which corresponded in structure to botogenin and neobotogenin.⁷ As shown in Table I, the melting points of the new sapogenins and their derivatives were decidedly different from those of the incompletely characterized "botogenin" series. Because of these differences we named the sapogenins gentrogenin (botogenin) and correllogenin.^{8a,b}

Gentrogenin and correllogenin were isolated by means of Girard's Reagent T from a crude sapogenin mixture also containing diosgenin and yamogenin. The isomers were best separated by fractional crystallization of their acetates from ethyl

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(2) Paper XXXIV, *J. Am. Chem. Soc.*, **78**, 1747 (1956).

(3) A preliminary report has appeared in *J. Am. Chem. Soc.*, **77**, 5196 (1955).

(4) Presented at Delaware Valley Regional meeting, AMERICAN CHEMICAL SOCIETY, Philadelphia, Pa., Feb. 16, 1956; and 129th National Meeting, AMERICAN CHEMICAL SOCIETY, Dallas, Tex., April 8-13, 1956.

(5) R. E. Marker and J. Lopez, *J. Am. Chem. Soc.*, **69**, 2397 (1947).

(6) (a) M. E. Wall *et al.*, *J. Am. Pharm. Assoc.*, **43**, 1 (1954). (b) M. E. Wall *et al.*, *J. Am. Pharm. Assoc.*, **43**, 503 (1954). (c) M. E. Wall *et al.*, *J. Am. Pharm. Assoc.*, **44**, 438 (1955).

(7) R. E. Marker, *J. Am. Chem. Soc.*, **71**, 2656 (1949).

(8) (a) These sapogenins were named in honor of Doctors H. S. Gentry and D. S. Correll, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Md., who obtained the *Dioscorea* samples from which the new sapogenins were obtained. (b) One of the reviewers feels that it was improper to change the earlier name botogenin to gentrogenin since the two sapogenins apparently have the same structure. The other reviewer feels that the renaming was justified. At present we are retaining both names gentrogenin (botogenin) until this issue can be further resolved.

TABLE I

Compound (Reported by Marker ^{6,7})	Melting Point		Compound
Botogenin	261-263	215-216	Gentrogenin
Botogenin acetate	246-248	227	Gentrogenin acetate
Neobotogenin	246-248	209-211	Correllogenin
Neobotogenin acetate	234	213-214	Correllogenin acetate
I ^a (From boto- genin)	226-228	173-175	I ^a (From gentro- genin)
III ^b	205-207	222-223	III ^b
V ^c	262-264	210-212	V ^c

^a I = 5,16-Pregnadien-3 β -ol-12,20-dione 3-acetate. ^b III = 5-Pregnen-3 β -ol-12,20-dione 3-acetate. ^c V = Allopregnane-3,12,20-trione.

acetate. In this manner the relatively insoluble gentrogenin acetate was easily separated from

et al.,^{9a} gentrogenin may be designated as 20 α ,22 α ,-25d-spirost-5-en-3 β -ol-12-one.¹⁰ The infrared spectrum of gentrogenin (*cf.* experimental section) was in accordance with the chemical data, showing the presence of a 12-ketone, nuclear unsaturation, and typical "22 α " (25d) fingerprint bands.^{11a,b}

Wolff-Kishner reduction of correllogenin gave the known yamogenin,⁹ thus establishing the compound as a Δ^5 -ketonic sapogenin of the 25L series. The infrared spectrum of correllogenin was in accord with these findings and indicated that the carbonyl was probably at C₁₂. Catalytic reduction of correllogenin acetate, followed by oxidation of the intermediate (which was not isolated) gave a compound which, from the method of preparation, infrared spectrum and close resemblance to the recently discovered sisalagenin,¹² we deduce to be the C₂₅ epimer of hecogenin. Accordingly, corello-

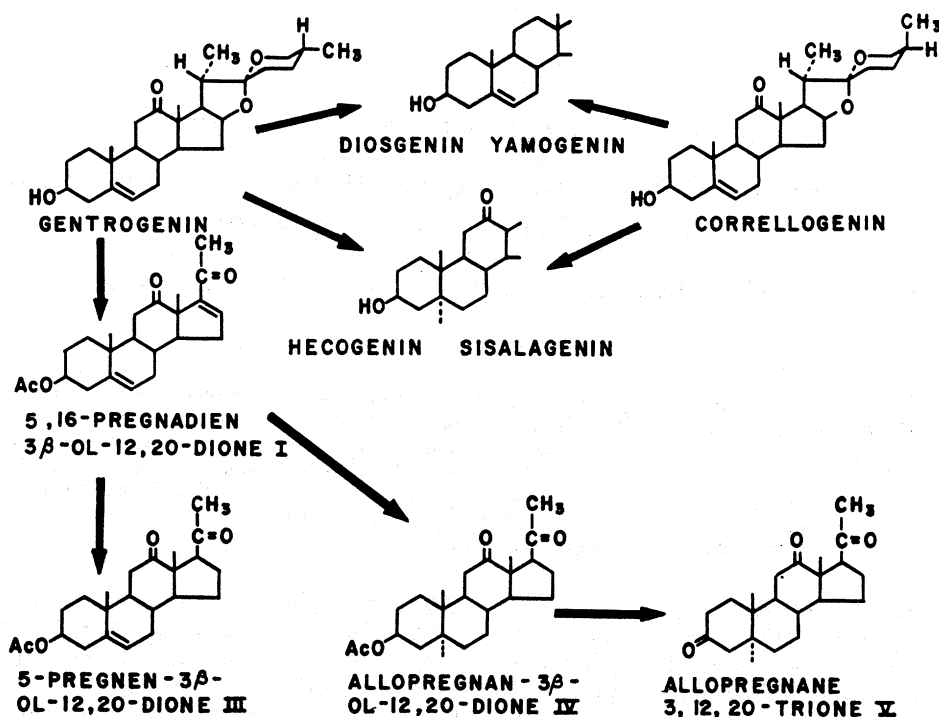


Fig. 1

correllogenin acetate. Purification of the latter was difficult and was accomplished only by chromatography of the mother liquors from gentrogenin, followed by repeated fractional crystallization.

Gentrogenin and correllogenin were characterized by the reaction sequence shown in Fig. 1. Wolff-Kishner reduction of gentrogenin gave diosgenin, thus establishing all salient features except the location of the carbonyl group. Catalytic hydrogenation of gentrogenin acetate with Adam's catalyst in ether containing 5% acetic acid gave yamogenin acetate which, on oxidation with chromium trioxide-acetic acid, yielded hecogenin. Since the structure of diosgenin was firmly established by Marker,⁹ and of hecogenin by Marker⁹ and Wagner

(9) R. E. Marker *et al.*, *J. Am. Chem. Soc.*, **69**, 2167 (1947).

(9a) R. B. Wagner, J. A. Moore, and R. F. Forker, *J. Am. Chem. Soc.*, **72**, 1856 (1950).

(10) The stereochemistry of the spiroketal side chain is still in question. Most workers now agree that naturally occurring sapogenins have the 20 α - configuration, are identical at C₂₂, and may occur as C₂₅ isomers. For leading references see Scheer, Kostic, and Mosettig, *J. Am. Chem. Soc.*, **77**, 641 (1955); Ziegler, Rosen, and Shabica, *J. Am. Chem. Soc.*, **77**, 1223 (1955); Wall, Serota, and Eddy, *J. Am. Chem. Soc.*, **77**, 1230 (1955); Hirschmann, Hirschmann, and Corcoran, *J. Org. Chem.*, **20**, 572 (1955); James, *J. Chem. Soc.*, 637 (1955); Callow *et al.*, *J. Chem. Soc.* 1966 (1955); Wall, *Experientia*, **11**, 340 (1955).

(11) (a) M. E. Wall, C. R. Eddy, M. L. McClennan, and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); (b) R. N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953).

(12) R. K. Callow and V. H. T. James, *J. Chem. Soc.*, 1671 (1955).

genin must be designated as 20 α ,22 α ,25L-spirost-5-en-3 β -ol-12-one.

Degradation of the side chain of gentrogenin in our usual manner¹³ gave 5,16-pregnadien-3 β -ol-12,20-dione 3-acetate (I). The melting point, 173–175°, was about 50° lower than the value reported by Marker.⁷ The ultraviolet and infrared spectrum of the compound exhibited the typical hypsochromic shifts noted previously with other 16-dehydro-12,20-pregnenes.^{13,14}

Catalytic hydrogenation of I with palladium-barium sulfate gave 5-pregnen-3 β -ol-12,20-dione 3-acetate (III), with a melting point considerably higher than the value found by Marker for the same compound (Table I). The infrared spectrum and optical rotation of III was in accord with the assigned structure. Alkaline hydrolysis of I in *t*-butanol gave 5,16-pregnadien-3 β -ol-12,20-dione (II). Catalytic hydrogenation in the presence of platinum oxide followed by oxidation with chromium trioxide-pyridine reagent gave allopregnane-3,12,20-trione (V). The properties of V agreed with those described by Wagner, Moore, and Forker.^{9a} These workers had noted a 60° discrepancy between the melting points of V prepared by them from hecogenin or desoxycholic acid and V prepared by Marker from hecogenin or botogenin.^{7,9} Similar catalytic reduction and oxidation of the 16-dehydropregnene acetate I gave the known allopregnane-3 β -ol-12,20-dione 3-acetate (IV) with properties identical to those found previously for the same compound prepared from hecogenin.¹³

Gentrogenin and corrollogenin have been found, to date, only in the tubers of *Dioscorea spiculiflora*. This species was found in southern Mexico near the Guatemalan border. The total sapogenin content of several collections has varied from 2–5.5%, dry basis. The ketonic fractions constituted 30–55% of the total sapogenins. Gentrogenin was predominant in this fraction; similarly diosgenin was predominant in the nonketonic fraction, yamogenin being a minor constituent.

We note that sapogenins of the 25L series are of rather infrequent occurrence in nature. It might be reasoned from conformational analysis that 25L sapogenins are less stable than 25D forms (cf. references in footnote 10). However, it would seem that specific plant enzyme systems for 25D or 25L configurations must be involved, for in some cases only 25L sapogenins are found.¹⁵

EXPERIMENTAL

Melting points were obtained with a Kofler micro melting-point apparatus. All optical rotations were determined

(13) M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Am. Chem. Soc.*, **77**, 5665 (1955).

(14) G. P. Mueller, R. E. Stobaugh, and R. S. Winniford, *J. Am. Chem. Soc.*, **75**, 4888 (1953).

(15) For example, in *Dioscorea bartlettii* only yamogenin is found. In a number of *Yucca* species, sarsapogenin is the only sapogenin found.

in chloroform solution. Ultraviolet spectra were obtained in methanol, infrared spectra in carbon disulfide.

Isolation of gentrogenin (botogenin) and corrollogenin. Tubers of *Dioscorea spiculiflora*, 21.9 kg., 40% moisture, were ground, extracted with isopropyl alcohol, and the crude sapogenins isolated in our usual manner.¹⁶ The crude sapogenins, 1.0 kg., were refluxed in 5 l. of acetic anhydride. The crystalline but crude sapogenin acetate mixture thus obtained weighed 470 g. It was heated with a mixture of 6 l. of absolute ethanol and 1.2 l. of glacial acetic acid. To the resultant solution was added 150 g. of Girard Reagent T and the mixture refluxed 0.5 hr. The solution was cooled to room temperature and slowly poured over a mixture of 11 kg. of ice and 1.02 kg. of sodium carbonate with continuous stirring. The mixture was diluted with 19 l. of water and extracted with three 10-l. portions of ether, thus removing nonketonic sapogenins. The ether was washed with water and the washings were added to the original aqueous fraction. The aqueous solution was strongly acidified with hydrochloric acid, heated for several hours, and allowed to stand overnight. The ketonic fraction was filtered and washed. After chromatography on Florisil, 45.0 g. of pure ketonic fraction was obtained. Similar chromatography of the nonketonic fraction gave 293 g. of a mixture of diosgenin and yamogenin. Infrared examination indicated that yamogenin was a minor fraction.

Gentrogenin, 20 α ,22 α ,25D-spirost-5-en-3 β -ol-12-one. The ketonic fraction obtained as described above was dissolved in hot ethyl acetate. The solution was allowed to come slowly to room temperature. Long rods formed which were filtered and crystallized several more times from ethyl acetate to give gentrogenin acetate, m.p. 227°, $[\alpha]_D^{25}$ –56°. The infrared spectrum showed two carbonyl peaks, 1735 cm.⁻¹ (acetate), 1712 cm.⁻¹ (12-ketone), a weak band at 836 cm.⁻¹ (Δ^6 ethylenic band ^{17a,b}), and the typical “22a”-25D fingerprint spectrum 980 (s), 919 (w), 898 (s), and 863 (w) cm.⁻¹,^{11a,b}

Anal. Calcd. for C₂₉H₄₂O₆: C, 74.01; H, 9.00. Found: C, 74.10; H, 9.10.

Hydrolysis of gentrogenin acetate in refluxing methanol containing 5% potassium hydroxide followed by the usual ether work-up, gave gentrogenin, rectangular plates from methanol, m.p. 215–216°, $[\alpha]_D^{25}$ –57°.

Anal. Calcd. for C₂₇H₄₀O₄: C, 75.66; H, 9.41. Found: C, 75.46; H, 9.51.

Diosgenin from gentrogenin. One-tenth g. of gentrogenin was submitted to the Huang-Minlon modification of the Wolff-Kishner reaction.¹⁸ After the usual work-up, crystallization from acetone gave 0.08 g. of diosgenin, m.p. 198–200°, infrared spectrum identical to an authentic reference sample.

Rockogenin and hecogenin from gentrogenin. Three-tenths g. of gentrogenin acetate was catalytically hydrogenated at 3 atmospheres pressure with 0.3 g. Adam's catalyst (platinum oxide) in ether containing 5% acetic acid. The product on crystallization from methanol had m.p. 215° with infrared spectrum identical to rockogenin 3-monoacetate prepared by similar reduction of hecogenin acetate. The total reduction product, 0.3 g., consisting of isolated crystals and mother liquors, was taken up in 100 ml. of acetic acid. To this solution, maintained at 25° by a water bath, was added dropwise a solution of 0.3 g. of chromium trioxide in 15 ml. of 80% acetic acid. The mixture was allowed to stand 1 hr. and then was given our usual work-up.¹⁹ Several crystal-

(16) M. E. Wall, M. M. Krider, E. S. Rothman, and C. R. Eddy, *J. Biol. Chem.*, **198**, 533 (1952).

(17) (a) R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **73**, 3215 (1951). (b) C. R. Eddy, M. E. Wall, and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(18) Huang-Minlon, *J. Am. Chem. Soc.*, **71**, 3301 (1949).

(19) For details of our work-up procedures, see *J. Am. Chem. Soc.*, **77**, 1230, 5665 (1955).

lizations from methanol gave 0.16 g. of hecogenin acetate, m.p. 245–247°, infrared spectrum identical to an authentic reference sample.

Correllogenin, 20 α ,22 α ,25 β -spirost-5-en-3 β -ol-12-one. The mother liquors from the ethyl acetate crystallization of gentrogenin acetate contained both correllogenin and gentrogenin acetates. Repeated crystallizations from ethyl acetate removed more gentrogenin acetate, leaving the soluble fractions enriched in correllogenin acetate. Chromatography on Florisil and silica gel was not particularly effective but gave a slight enrichment of correllogenin acetate in the more polar eluates. The residues from these treatments were crystallized repeatedly from ethyl acetate-methanol and finally methanol to give correllogenin acetate, needles from methanol, m.p. 213–214°, $[\alpha]_D^{25}$ –60°, infrared spectrum similar to gentrogenin acetate but showed typical 25 μ fingerprint bands^{11a,b}, 986 (s), 920 (s), 897 (w), and 852 (w) cm.⁻¹

Anal. Calcd. for C₂₈H₄₂O₅: C, 74.01; H, 9.00. Found: C, 73.90; H, 9.18.

Hydrolysis of the acetate gave correllogenin, m.p. 209–211°, $[\alpha]_D^{25}$ –69°.

Anal. Calcd. for C₂₇H₄₀O₄: C, 75.66; H, 9.41. Found: C, 75.14; H, 9.63.

Yamogenin from correllogenin. Wolff-Kishner reduction of correllogenin gave yamogenin, m.p. 187–189°, infrared spectrum identical to an authentic specimen.

Sisalagenin from correllogenin. Catalytic hydrogenation of correllogenin acetate followed by chromium trioxide oxidation in the same manner described for gentrogenin gave a product²⁰ which we believe is the recently isolated sisalagenin acetate,¹³ m.p. 214–216°, $[\alpha]_D^{25}$ –12° (lit.¹² gives m.p. 228–232°, $[\alpha]_D^{25}$ –12°), infrared spectrum showed peaks at 1733 (s), 1712 (s), 1071 (s), 1037 (s), 987 (s), 919 (s), 899 (w), 849 (w) cm.⁻¹ which were in agreement with data of Callow and James.¹²

5,16-Pregnadien-3 β -ol-12,20-dione 3-acetate (I). Eight g. of gentrogenin acetate was refluxed 5 hr. in 40 ml. of acetic anhydride to which was added 1.9 g. of pyridine hydrochloride. The crude pseudogentrogenin diacetate thus obtained was oxidized in our usual manner¹² and the oxidation intermediate treated with potassium hydroxide in *t*-butyl alcohol.¹² After the standard work-up, the product was acetylated and chromatographed on Florisil. Elution with benzene and chloroform followed by crystallization from

ether gave 2.3 g. of I, m.p. 170–173°. The analytical sample after 3 ether crystallizations had m.p. 173–175°, $[\alpha]_D^{25}$ +57°, λ_{max}^{OH} 227.5 m μ , log ϵ 3.98; $\nu_{max}^{C=O}$ 1737, 1720, and 1684 cm.⁻¹.

Anal. Calcd. for C₂₈H₄₀O₄: C, 74.56; H, 8.16. Found: C, 74.35; H, 8.20.

Alkaline hydrolysis of I in *t*-butyl alcohol-potassium hydroxide gave 5,16-pregnadien-3 β -ol-12,20-dione (II), needles from ethyl acetate, m.p. 198–202°, $[\alpha]_D^{25}$ +67.4°.

5-Pregnen-3 β -ol-12,20-dione 3-acetate (III). Two-tenths g. of I was dissolved in 100 ml. of ethanol and catalytically hydrogenated in the presence of 0.4 g. of 10% palladium on barium sulfate at 3 atmospheres for 16 hr. After filtration and removal of the ethanol the residue was crystallized from methanol, long rods, m.p. 222–223°, $[\alpha]_D^{25}$ +90.4°, infrared spectrum shows absence of conjugated carbonyl, and presence of two strong carbonyl bands, 1735 cm.⁻¹ (acetate), 1710 cm.⁻¹ (C₁₂ and C₂₀ carbonyl).

Allopregnane-3 β -ol-12,20-dione 3-acetate (IV). One-tenth g. of I in 47 ml. of ether containing 3 ml. of glacial acetic acid was catalytically hydrogenated in the presence of 0.1 g. of platinum oxide at 3 atmospheres pressure for 16 hr. After the usual work-up, the residual glass was oxidized in chromium trioxide-pyridine.²¹ Dilution with water, and ether extraction gave a crude product which was taken up in a small volume of methylene chloride to which was added a large volume of ether. The solution was concentrated on the steam bath. On standing, 30 mg. of crystalline product was obtained, m.p. 190–192° with infrared spectrum identical to an authentic specimen of IV from hecogenin.¹³

Allopregnane-3,12,20-trione (V). Two-tenths g. of II were reduced and oxidized as described under IV. The crude product was chromatographed on Florisil and the benzene and chloroform eluates were combined and triturated with ether. The ether-insoluble residue was crystallized from ethyl acetate as irregular plates, m.p. 210–212° (lit.^{9a} gives m.p. 207–208°), infrared spectrum identical with that of an authentic specimen derived from hecogenin.

Acknowledgment. We wish to thank R. F. Mininger for optical rotation data and K. Zbinden for C and H analyses. The infrared spectra were obtained by C. S. Fenske under the supervision of C. R. Eddy.

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(21) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

(20) We were unable to obtain enough pure correllogenin acetate to purify adequately the reduction product or obtain analytical data.